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IAP9 Rec'd PCT/PTO 24 MAY 2006

PLATINUM(II) COMPLEXES, PREPARATION AND USE

BACKGROUND OF THE INVENTION

THIS invention relates to the preparation of platinum(II) complexes, in particular the preparation of dicarboxylatoplatinum(II) complexes containing a neutral bidentate ligand, (such as oxaliplatin, which has become increasingly important due to its anti-cancer activity).

Dicarboxylatoplatinum(II) complexes (such as oxaliplatin) containing a neutral bidentate ligand ("non-leaving group") have in the past been synthesized by way of a process that utilizes a silver salt to remove halide ions from the complex. The use of a silver compound in the process results

in numerous contaminants, which must be removed by further processes in order to achieve purity that is suitable for anti-cancer pharmaceutical agent purposes.

Oxaliplatin and its pharmaceutical properties were first disclosed by Kidani et al. in J Med Chem, 1978, 21, 13135 and in United States Patent No. 4,169,846. In this patent a halogenoplatinum compound is used as the starting material. Halide ions are removed by a silver salt, whereafter an oxalate is introduced either as the free acid or a salt thereof.

In general, a method for the production of oxaliplatin is as set out below:

Step 1.

$1\text{mol } K_2PtX_4 + 1\text{mol } L \rightarrow PtLX_2$

X = Cl, Br, I and L = *trans*-ℓ-1,2-diaminocyclohexane

Step 2.

$1\text{ mol } PtLX_2 + 2\text{ mol } AgNO_3 \rightarrow PtL(H_2O)_2^{2+} + 2AgX + 2NO_3^-$

or

$1\text{ mol } PtLX_2 + 1\text{ mol } Ag_2Y \rightarrow PtL(H_2O)_2^{2+} + 2AgX + Y^{2-}$

$Y = SO_4^{2-}$

Step 3.

$PtL(H_2O)_2^{2+} + Z_2(\text{oxalate}) \rightarrow PtL(\text{oxalate}) + 2Z^+$

$Z = K^+, Na^+$ or H^+

US Patent No. 5,290,961 in the name of Tanaka Kikinzoku Kogyo K.K. teaches that the abovementioned method has the disadvantage that many impurities are incorporated into the products. These impurities include unreacted $PtLX_2$, AgX and Ag^+. The presence of $PtLX_2$ is attributed to their generally insoluble nature in water. As a result, large quantities of water

must be used in step 2 to dissolve $PtLX_2$. This prevents the AgX, even though it is insoluble in water, from being completely removed from the solution. US patents nos. 5,338,874 and 5,420,319, also in the name of Tanaka Kikinzoku Kogyo K.K., teach processes for the production of cis-oxalato(*trans-ℓ*-1,2-cyclohexanediamine)platinum(II) with high optical purity which can be used as an active pharmaceutical ingredient of a carcinostatic agent. However, these processes also follow complicated multi-step pathways, making use of silver compounds which must also ultimately be removed from the process.

SUMMARY OF THE INVENTION

The present invention has been made to address the drawbacks of the prior art, in terms of which dicarboxylatoplatinum(II) complexes containing a neutral bidentate ligand, such as oxaliplatin, are produced using the silver method, which results in contaminants in the final product, is time consuming and expensive. Furthermore, the absence of silver compounds in the process enables the synthesis of a group of novel platinum compounds i.e ligands containing donor atoms other than N such as S or Se.

A first aspect of the invention relates to a method for the preparation of a platinum(II) complex, in particular a dicarboxylatoplatinum(II) complex containing a neutral bidentate ligand, such as oxaliplatin, the method including the step of reacting a *bis*-dicarboxylatoplatinate(II) species with a suitable neutral bidentate ligand to form a neutral dicarboxylatoplatinum(II) complex and if necessary recrystallising the product to form a pure dicarboxylatoplatinum(II) complex containing a neutral bidentate ligand.

The *bis*-oxalatoplatinate(II) species and ligand are typically reacted at a temperature of 40°C to 100°C, preferably approximately 95°C, for a period of 0.5 to 3 hours, preferably 1 hour.
Any dicarboxylatoplatinate(II) species may be removed from the product by

dissolving the product in distilled water and adding an oxalate such as $Cs_2C_2O_4$, which transforms the dicarboxylatoplatinate(II) species into a species that can be separated from the dissolved product by filtration.

The neutral bidentate ligand is typically an amine.

The amine may be a diamine.

Where the method is for the preparation of chemically and optically pure oxaliplatin, the ligand is optically pure trans-ℓ-1,2-diaminocyclohexane.

The neutral bidentate ligand may contain donor atoms other than N, or N together with a donor atom other than N, typically S and Se, for example:

- neutral bidentate heterocyclic amines with an S donor atom (for example thioetherial groups), such as:

1-alkyl/aryl-2-alkylthioalkyl/aryl heterocyclic amines, particularly imidazoles or pyridines, for example:

Ligand (i) 1-methyl-2-methylthioethylimidazole
Ligand (ii) 1-methyl-2-methylthiopropylimidazole
Ligand (iii) 1-butyl-2-methylthiomethylimidazole
Ligand (iv) 1-methyl-2-methylthiomethylimidazole
Ligand (v) 1-butyl-2-methylthioethylimidazole
Ligand (vi) 2-methylthiomethylpyridine
Ligand (vii) 2-methylthioethylpyridine
Ligand (viii) 2-methylthiopropylpyridine;

- aminoalkylthioalkyl/aryl compounds for example:

Ligand (ix) 1-amino-2-thiomethylethane
Ligand (x) 1-amino-2-thioethylethane;

- dithioethers for example:

Ligand (xi) 2,5-dithiahexane;

- diseleno ethers for example:

Ligand (xii) 2,5-diseleno hexane; etc.

New oxalatoplatinum(II) complexes containing S or Se donor atoms that can be prepared using the method of the invention include:

Complex (i) oxalato(1-methyl-2-methylthioethylimidazole)platinum(II)
Complex (ii) oxalato(1-methyl-2-methylthiopropylimidazole)platinum(II)
Complex (iii) oxalato(1-butyl-2-methylthiomethylimidazole)platinum(II)
Complex (iv) oxalato(1-methyl-2-methylthiomethylimidazole)platinum(II)
Complex (v) oxalato(1-butyl-2-methylthioethyimidazole)platinum(II)
Complex (vi) oxalato(2-methylthiomethylpyridine)platinum(II)
Complex (vii) oxalato(1-amino-2-thioethylethane)platinum (II)
Complex (viii) oxalato(1-amino-2-thiopropylethane)platinum (II)
Complex (ix) oxalato(1-amino-2-thiomethylethane)platinum(II)
Complex (x) oxalato(1-amino-2-thioethylethane)platinum(II)
Complex (xi) oxalato(2,5-dithiahexane)platinum(II)
Complex (xii) oxalato(2,5-diseleno hexane)platinum(II).

The above new complexes may be used in methods of treating cancer in patients, and in methods of manufacturing medicaments for treating cancer in patients

The complexes produced according to the method of the invention contain no traces of silver.

A second aspect of this invention is a method for producing a *bis*-dicarboxylatoplatinate(II) species, e.g. a *bis*-oxalatoplatinate(II) salt which may be used in the method of the first aspect of the invention. The method according to the second aspect of the invention includes the step of either reacting a platinum(II) compound, such as K_2PtX_4 or reacting a platinum(IV) compound such as K_2PtX_6 where X is a halide such Cl, Br or I, preferably Cl, with a dicarboxylate such as an oxalate, wherein the platinum(II) or platinum(IV) compound and oxalate salt are reacted at a high mole ratio of greater than 1:4, preferably 1:8 or greater, more preferably 1:16 or greater most preferably1:24 or greater.

In the case of the platinum(IV) compound, this compound is reduced to platinum(II) by the oxalate, or it may be reduced by another reducing agent such as SO_2 or sulfite.

The oxalate is typically $K_2C_2O_4$.

The platinum(II) *bis*-dicarboxylato species is typically $K_2Pt(C_2O_4)_2.2H_2O$.

The platinum(II) compound or platinum(IV) compound and oxalate are typically reacted at a temperature of from 40°C to less than 100°C, preferably approximately 95°C, for a period of 0.5 to 4 hours, typically 1 hour.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph indicating the efficiency of the synthesis of $K_2Pt(C_2O_4)_2.2H_2O$ relative to the ratio of $K_2C_2O_4$ to K_2PtCl_6 in a reaction of K_2PtCl_6 with $K_2C_2O_4$ with a constant reaction time of 1h 15 min at 95°C;

Figure 2 is a graph indicating the time taken to reach an 85% yield of $K_2Pt(C_2O_4)_2.2H_2O$ relative to the oxalate to platinum ratio in the reaction of $K_2C_2O_4$ with K_2PtCl_6 at 95°C;

Figure 3 is a chromatographic analysis of the oxaliplatin product which did not dissolve when suspended in 6ml water, in Example 4;

Figure 4 is a chromatographic analysis of the oxaliplatin product of Figure 3 which has subsequently been washed with water, in Example 4; and

Figure 5 is a chromatographic analysis of the chemically pure oxaliplatin product of Example 4 as determined on a chiral column indicating its optical purity.

DESCRIPTION OF PREFERRED EMBODIMENTS

This invention relates to a method for the preparation of platinum(II) complexes, in particular a dicarboxylatoplatinum(II) complex containing a neutral bidentate ligand ("non-leaving group") such as oxaliplatin, and new platinum(II) complexes that include a donor atom other than N, the method includes the steps of:

Step 1. the production of a *bis*-dicarboxylatoplatinate(II) species, typically $K_2Pt(C_2O_4)_2.2H_2O$;

Step 2. reaction of the *bis*-dicarboxylatoplatinate(II) species with a suitable neutral bidentate ligand to form a dicarboxalatoplatinum(II) complex containing a neutral bidentate ligand product; and if necessary

Step 3. recrystallising the product to form a pure oxalatoplatinum(II) complex containing a neutral bidentate ligand product.

Step 1

The reaction times quoted in all of these steps are given for reagent quantities of ~10 g.

A *bis*-dicarboxylatoplatinate(II) species such as $K_2Pt(C_2O_4)_2.2H_2O$ may be synthesized using either K_2PtCl_6, or K_2PtCl_4 as starting materials. It may be possible to use H_2PtCl_6 as a starting material.

Method 1 – synthesis of $K_2Pt(C_2O_4)_2.2H_2O$ using K_2PtCl_4

K_2PtCl_4 and $K_2C_2O_4$ may be dissolved in distilled water in the mole ratio of

1:16 and stirred at a temperature of between 40°C to 100°C, typically approximately 95°C for 0.5 to 2 hours, typically 1 hour. The solution is refrigerated at a temperature from 2°C to 10°C, typically approximately 5°C for 0.5 to 2 hours typically 1 hour to complete crystallization where after it may be filtered. The precipitated product may be washed 5 times with small volumes of cold water and once with acetone and allowed to air dry.

1 mol K_2PtCl_4 + 16 mol $K_2C_2O_4$ $\rightarrow$ $K_2Pt(C_2O_4)_2.2H_2O$

Method 2 – synthesis of $K_2Pt(C_2O_4)_2.2H_2O$ using K_2PtCl_6 (oxalate acting as reducing and complexing agent)

$K_2C_2O_4$ and K_2PtCl_6 may be suspended in water in a mole ratio of 34:1 and stirred at a temperature of between 40°C to 100°C, typically approximately 95°C for 0.5 to 4 hours typically 55 minutes as illustrated by the curve in Figure 2. Only 10 minutes additional time is required for a ratio of 24:1. The reaction vessel may be maintained at a temperature between 2°C to 10°C, typically approximately 5°C for 0.5 to 2 hours typically 1 hour to allow for complete crystallization followed by filtration. The precipitate may be washed 5 times with small volumes of cold water and once with acetone and allowed to air dry.

1 mol K_2PtCl_6 + 34mol $K_2C_2O_4$ $\rightarrow$ $K_2Pt(C_2O_4)_2.2H_2O$

In this reaction the oxalate plays two roles. Firstly, it acts as a reducing agent. On heating the solution of $K_2C_2O_4$ with a suspension of K_2PtCl_6 the latter dissolves to form a dark orange solution with the evolution of gas. The disappearance of the starting material accompanied with a darkening of the solution indicates the formation of $PtCl_4^{2-}$ therefore a reduction of platinum(IV) to platinum(II). The evolution of gas further indicates the oxidation of oxalate to CO_2.

After all the K_2PtCl_6 has dissolved, the oxalate starts a second role where it

acts as a complexing agent and a light yellow precipitate starts to form. Thus the oxalate acts as a suitable complexing agent which can coordinate to the platinum to form $K_2Pt(C_2O_4)_2 \cdot 2H_2O$. Therefore, this method can be divided into two separate reactions. An alternative reducing agent such as SO_2 may be used in the place of the oxalate, to reduce the platinum IV to platinum II.

$$K_2PtCl_6 \xrightarrow[K_2C_2O_4]{} K_2Pt(C_2O_4)_2.2H_2O$$

In prior art methods which describe the synthesis of $K_2Pt(C_2O_4)_2.2H_2O$, a small excess of $K_2C_2O_4$ of up to 4 times was used at a temperature of 100°C for an extensive period (up to 18 hrs). See Shriver DF (Ed) 1979. Inorganic Synthesis, Vol. XIX: 16-17. During that time reduction of the platinum species occurs forming platinum metal (platinum black).

In accordance with an aspect of the method of this invention, the inventor has quite unexpectedly found out that when a platinum compound and oxalate are reacted at a high mole ratio of greater than 1:4, preferably greater than 1:8, more preferably greater than 1:16, most preferably 1:24 or greater and lower reaction temperatures (less than 100°C, typically 95°C), shorter reaction times are attained and no reduction to platinum metal (no platinum black) is observed. The higher concentration of the complexing anion, oxalate, not only acts as a stabilizater of the *bis*-oxalatoplatinate(II) species but also improves reaction rates of reduction as well as ligand exchange thus resulting in high yields of the *bis*-oxalatoplatinate(II) species. The larger the excess oxalate used, the higher the percentage yield of $K_2Pt(C_2O_4)_2.2H_2O$. (See Figure 1), when a 1:16 ratio of $K_2C_2O_4$ is used relative to K_2PtCl_6, the yield of $K_2Pt(C_2O_4)_2.2H_2O$ is only 67%. The yield consistently increases as the oxalate excess increases such that a ratio of 34:1 results in a 86% yield (See Figure 2). In Figure 2 the ratio of platinum to potassium oxalate is plotted against the time in minutes required to reach the maximum yield of the production of $K_2Pt(C_2O_4)_2.2H_2O$ from K_2PtCl_6, namely ~85%. Experiments performed with ratios of 8:1 or lower resulted

in the formation of finely divided black platinum metal due to decomposition. This gradually occurs upon heating for approximately 8½ hours. When ratios of 3:1-8:1 are employed, extensive reaction time periods are required to reach maximum efficiencies of conversions, which still results in low yields. In the literature, a ratio of 3:1 results in a 30% yield after refluxing for 18 hours (1080mins) (Synthesis of $K_2Pt(C_2O_4)_2.2H_2O$, Shriver, D.F., Inorganic Synthesis, 19, pp.16-17, (ed.). 1979.). As the amount of oxalate increases, the time taken to reach the 85% yield decreases from 156 mins at a mole ratio 12:1, to 110 mins at a mole ratio of 16:1, to 65 mins at a mole ration of 24:1 to 55 mins at a mole ratio of 34:1.

Step 2

The *bis*-dicarboxylatoplatinate(II) species viz. $K_2Pt(C_2O_4)_2.2H_2O$, may be reacted with a suitable, preferably optically pure, neutral bidentate ligand to form a neutral platinum(II) oxalato compound such as oxaliplatin.

$K_2Pt(C_2O_4)_2.2H_2O$ may be dissolved in a suitable solvent system whereafter a ligand (L) dissolved in a suitable solvent is added. The ligand may be selected from any bidentate neutral donor ligand, but in the case of oxaliplatin is a diamine, namely optically pure *trans-ℓ*-1,2-diaminocyclohexane. Optically pure *trans-ℓ*-1,2-diaminocyclohexane may be obtained in an optically pure state by crystallization with tartaric acid, for example by a method described by Hans-Jörg Schanz, Michael A. Linseis and Declan G. Gilheany, in Improved resolution methods for (R,R)- and (S,S)-cyclohexane-1,2-diamine and (R)- and (S)-Binol. Tetrahedron Asymmetry 12 (2003), 2763-2769, the content of which is incorporated herein by reference. The solution may be maintained at a temperature from 40 to 100°C, typically approximately 95°C for 0.5 to 2 hours, typically 1 hour to form a precipitate which contains oxalatoplatinum(II) complex such as oxaliplatin.

$$1\text{mol } K_2Pt(C_2O_4)_2.2H_2O + 1\text{mol L} \rightarrow PtL(C_2O_4) + K_2C_2O_4$$

Step 3

The crude product may be purified by extracting the oxalatoplatinum(II) complex with sufficient excess of water. Contaminating $K_2Pt(C_2O_4)_2 \cdot 2H_2O$ which has similar solubility properties to the oxalatoplatinum(II) complex such as oxaliplatin at low temperatures may be removed by transforming it into $Cs_2Pt(C_2O_4)_2$ through addition of $Cs_2C_2O_4$ which upon cooling removes $Pt(C_2O_4)_2^{2-}$. The filtrate of this solution upon vacuum evaporation leaves a solid which can be washed with a small portion of hot water removing the residual amounts of $Pt(C_2O_4)_2^{2-}$ and oxalate salts. The white solid may be washed with cold water to obtain pure oxaliplatin. A further amount of oxalatoplatinum(II) complex may be obtained from the filtrate after removing $Cs_2Pt(C_2O_4)_2$ which precipitates after cooling. The final step consists of the recrystallization of the above white precipitate. A final oxaliplatin product has a chemical purity of >99.5% and optical purity of >99.98%. The overall yield of chemically and optically pure oxaliplatin is 15%.

Thus, the above method of the invention when used for producing oxaliplatin uses only 5 steps with an overall reaction time of 16 hours. It also requires the use of only four different chemicals, namely: K_2PtCl_6 / K_2PtCl_4, $K_2C_2O_4$, $Cs_2C_2O_4$ and a suitable neutral bidentate ligand.

The method described above may be used to form many other platinum(II) complexes with neutral bidentate ligands, and makes it possible to form platinum(II) complexes with neutral bidentate ligands that contain donor atoms other than N, typically S and Se, for example:

- neutral bidentate heterocyclic amines with an S donor atom, such as thioetherial S containing compounds of the general formula:

1-alkyl/aryl-2-alkylthioalkyl/aryl heterocyclic amines, particularly imidazoles or pyridines;

- aminoalkylthioalkyl/aryl compounds;
- dithioethers for example 2,5-dithiahexane;

- diseleno ethers for example 2,5-diseleno hexane; etc.

Ligands containing S and Se donor atoms cannot be used in reactions that make use of silver compounds, because these atoms react by binding very strongly with both platinum and silver ions.

The following 2-methylthioalkyl imidazole and pyridine neutral bidentate ligands :

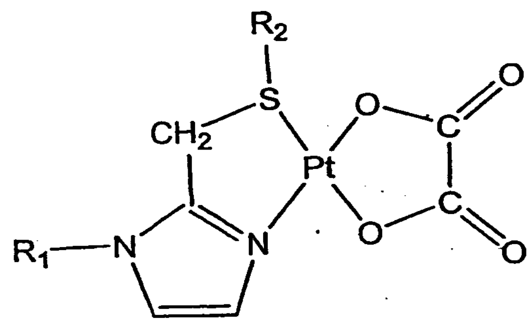
Ligand (i)	1-methyl-2-methylthioethylimidazole
Ligand (ii)	1-methyl-2-methylthiopropylimidazole
Ligand (iii)	1-butyl-2-methylthiomethylimidazole
Ligand (iv)	1-methyl-2-methylthiomethylimidazole
Ligand (v)	1-butyl-2-methylthioethylimidazole
Ligand (vi)	2-methylthiomethylpyridine
Ligand (vii)	2-methylthioethylpyridine
Ligand (viii)	2-methylthiopropylpyridine

(prepared by the methods described in JGH du Preez, TIA Gerber, W Edge, VLV Mtotywa and BJAM van Brecht. Nitrogen Reagents in Metal Ion Separation. XI. The Synthesis and Extraction Behaviour of a New NS imidazole Derivative. Solv. Extr. & Ion Exch. (2001) 19(1), 143-154) (the content of which is incorporated herein by reference) may be used in the below method to prepare the 2-methylthioakyl complexes of imidazole and pyridine (i) to (v) mentioned below.

$K_2Pt(C_2O_4)_2.2H_2O$ may be dissolved in distilled water over a 90°C water bath to which is added dropwise while stirring one molar equivalent of the relevant neutral bidentate ligand dissolved in acetone. The platinum(II) solution so formed may be stirred for 1½ hours at 90°C and subsequently allowed to cool. The resultant precipitate may be filtered and washed once with cold distilled water and air dried in an oven at 50°C.

Examples of 2-methylthioalkyl complexes of imidazole prepared by the above method are reflected in the structural Formula (I) below where R_1

and R_2 may be selected from alkyl (e.g. CH_3, C_2H_5 etc.) and aryl (e.g. phenyl) groups. Typical 2-methylthioakyl complexes of imidazole are complexes (i) to (v) below:



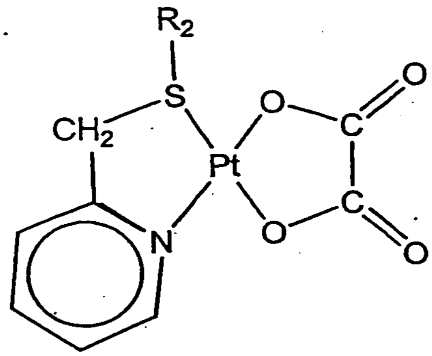
Formula (I)

Complex (i)	$R_1 = CH_3$	$R_2=C_2H_5$
Complex (ii)	$R_1 = CH_3$	$R_2=C_3H_7$
Complex (iii)	$R_1 = C_4H_9$	$R_2= CH_3$
Complex (iv)	$R_1 = CH_3$	$R_2= CH_3$
Complex (v)	$R_1 = C_4H_9$	$R_2= C_2H_5$

The chemical names for the complexes (i) to (v) are:

Complex (i)	oxalato(1-methyl-2-methylthioethylimidazole)platinum(II)
Complex (ii)	oxalato(1-methyl-2-methylthiopropylimidazole)platinum(II)
Complex (iii)	oxalato(1-butyl-2-methylthiomethylimidazole)platinum(II)
Complex (iv)	oxalato(1-methyl-2-methylthiomethylimidazole)platinum(II)
Complex (v)	oxalato(1-butyl-2-methylthioethyimidazole)platinum(II).

Examples of 2-methylthioalkyl complexes of pyridine of the invention are reflected in the structural Formula (II) below where R_2 may be selected from alkyl (e.g. CH_3, C_2H_5 etc.) and aryl (e.g. phenyl) groups. Typical 2-methylthioalkyl complexes of pyridine are compounds (vi) to (viii) below:



Formula (II)

Complex (vi) $R_2 = CH_3$
Complex (vii) $R_2 = C_2H_5$
Complex (viii) $R_2 = C_3H_7$

The chemical names for the complexes (vi) to (viii) are:
Complex (vi) oxalato(2-methylthiomethylpyridine)platinum(II)
Complex (vii) oxalato(2-methylthioethylpyridine)platinum(II)
Complex (viii) oxalato(2-methylthiopropylpyridine)platinum(II).

2-methylthioakyl complexes of imidazole and pyridine mentioned above have been shown to have anti-cancer properties.

The following ligands:
Ligand (ix) 1-amino-2-thiomethylethane
Ligand (x) 1-amino-2-thioethylethane
may be used to prepare an aliphatic aminothioether complex of Pt(II)oxalate, using the method below:

$K_2Pt(C_2O_4)_2.2H_2O$ may be dissolved in distilled water over a 90°C water bath. Dimethylformamide (dmf) may be added to the platinum(II) solution to form a 20:80 water:dmf mixed solvent ratio. One molar equivalent of the relevant ligand may be dissolved in acetone and added dropwise while stirring whereafter the platinum(II)solution so formed may be stirred for 2 hours at 90°C. The reaction may be allowed to cool at room temperature

and the resultant precipitate filtered, washed once with cold distilled water and allowed to air dry in an oven at 50°C to produce a light yellow product (47% yield).

Examples of aliphatic aminothioether complexes of Pt(II)oxalate prepared by the above method are:

Complex (ix) oxalato(1-amino-2-thiomethylethane)platinum(II)

Complex (x) oxalato(1-amino-2-thioethylethane)platinum(II).

The conventional method of preparing oxaliplatin, such as the method described in US 5,420,319, uses 6 steps and has an overall reaction time of 38 hours. The method of the present invention reduces the reaction time and has fewer steps. Thus, the method of the invention is simpler, more efficient and more cost-effective than conventional methods. The method of the invention also eliminates the contamination problems experienced in conventional methods, such as that described in US 5,420,319. No $PtLX_2$ is formed which is generally quite insoluble in water and no silver is used which needs to be removed. The only by-products that need to be eliminated from the method of the invention is excess $K_2C_2O_4$ when synthesizing $K_2Pt(C_2O_4)_2.2H_20$ and unreacted $K_2Pt(C_2O_4)_2.2H_2O$ and oxalate salts when producing $PtL(C_2O_4)$ in the case of oxaliplatin. The excess oxalate salts are very soluble in water and can be removed easily by washing with distilled water.

A further advantage of not using a silver compound is that this reaction need not be carried out in darkness and can be used with neutral bidentate ligands that contain donor atoms other than N, such as S and Se which readily react with silver ions. Such ligands cannot be used in the methods that make use of silver compounds, thus preventing the synthesis of their carboxylato analogues. Thus, the method of the invention makes possible, *inter alia*, the synthesis of bidentate N,N ligand oxalato complexes of platinum(II) and a variety of new oxalatoplatinum(II) complexes containing bidentate ligands with N, S or Se donor atoms. These compounds, according to test results, have application as new anticancer agents which

may be used in methods of treating cancer in patients, and in methods of manufacturing medicaments for treating cancer in patients.

The invention will now by described with reference to the following non-limiting examples.

Example 1 – Production of $K_2Pt(C_2O_4)_2.2H_2O$ from K_2PtCl_4.

2.075g (4.6 mmole) K_2PtCl_4 and 13.561g (73.6 mmole) $K_2C_2O_4$ was dissolved in 30ml distilled water and stirred for 1 hour at approximately 95°C. The reaction vessel was subsequently refrigerated at approximately 5°C for 2 hours and the precipitate filtered, washed 5 times with 4ml water, rinsed with 2ml acetone and air dried in a 50°C oven. The yield was 98%.

Example 2 – Production of $K_2Pt(C_2O_4)_2.2H_2O$ from K_2PtCl_6 with oxalate as the reducing agent.

1.425g (2.9 mmole) K_2PtCl_6 and 18.392g (99.8 mmole) $K_2C_2O_4$ was dissolved in 50ml distilled water at approximately 95°C and stirred for 1 hour 15 minutes, subsequently cooled in a refrigerator for 2 hours and filtered. The excess $K_2C_2O_4$ was removed by washing the precipitate 5 times with 3ml portions of water and rinsed once with 2ml acetone. It was allowed to air dry in a 50°C oven. The yield was 86%.

Example 3 - Production of $K_2Pt(C_2O_4)_2.2H_2O$ from K_2PtCl_6 with SO_2 as the reducing agent.

0.326g (0.67 mmole) K_2PtCl_6 was suspended in 6ml distilled water at approximately 95°C. A saturated SO_2 solution was added drop wise till all the K_2PtCl_6 dissolved. Excess $K_2C_2O_4$ (1.981g (10.8 mmole) was added directly to the platinum solution and stirred for a further hour. The mixture was refrigerated for 2 hours and filtered. The precipitate was washed 5 times with 3ml portions of water, rinsed once with 1ml acetone and allowed

to air dry in a 50°C oven. The yield was 78%.

Example 4 - Synthesis of oxaliplatin.

$K_2Pt(C_2O_4)_2.2H_2O$ (4.50g, 9.97 mmol) was dissolved in distilled water (815ml) at 90°C to provide a platinum oxalate solution. One equivalent of optically pure trans-ℓ-1,2-diaminocyclohexane (produced by a method as described in Hans-Jörg Schanz, Michael A. Linseis and Declan G. Gilheany, Improved resolution methods for (R,R)- and (S,S)-cyclohexane-1,2-diamine and (R)- and (S)-Binol. Tetrahedron Asymmetry 12 (2003), 2763-2769) (1.06g, 9.27mmol) was dissolved in distilled water (74ml) and the pH adjusted with dilute acetic acid to between 6.6-7.5, to provide an amine solution. The amine solution was added incrementally in eight equal portions every 45 minutes to the platinum oxalate solution over 6 hours where after the solution was cooled to room temperature and the precipitate filtered. The solvent was removed from the filtrate under vacuum. The solid obtained from the filtrate contains oxaliplatin in a crude form.

This solid was suspended in distilled water (40ml) over a water bath at 70°C for a period of time (10 minutes) where after the remaining solid was removed by filtration. The filtrate was cooled and 0.75 molar equivalents of $Cs_2C_2O_4$ in relation to $K_2Pt(C_2O_4)_2.2H_2O$ was added. The solution was stirred for approximately 20 minutes and the precipitate which formed was filtered. The solvent was subsequently vacuum evaporated. The remaining solid was suspended in a small portion of distilled water (6ml) and heated to 70°C for a period of time (10 minutes) and filtered to provide a filtrate (A). The solid which did not dissolve contained mostly oxaliplatin with a small portion of oxalate salt (see Figure 3). This was readily purified by washing the solid with a small portion (3ml) of distilled water to obtain pure oxaliplatin (see Figure 4).

The filtrate (A) was cooled to 5°C and the precipitate filtered. The solvent was vacuum evaporated. The solid which remained contained mainly

oxaliplatin and cesium and potassium oxalates. The oxalate salts were readily removed by washing 5 times with small portions of distilled water (3ml) resulting in a further amount of pure oxaliplatin.

All the oxaliplatin samples produced had a chemical purity >99.5% (see Figure 4) and optical purity of >99.98% (see Figure 5).

The overall yield of chemically and optically pure oxaliplatin was 15%.

Example 5 – Method for producing oxaliplatin with K_2PtCl_4 as the starting material.

Both K_2PtCl_4 (41.5g; 92.0 mmole) and 271.17g (1178.8 mmole) of $K_2C_2O_4$ were simultaneously dissolved in 600ml water and kept at 95°C for at least 1 hour, during which time $K_2Pt(C_2O_4)_2.2H_2O$ gradually precipitated. The solution was cooled for over 2 hours at 5°C, filtered and washed with 5 80 ml portions of water and finally with acetone. After 45 minutes in an oven at 50°C pure dry $K_2Pt(C_2O_4)_2.2H_2O$ (43.72g; 89.96 mmole) was obtained. The product was dissolved in 120ml pure water at 95°C and *trans-ℓ*-1,2-diaminocyclohexane (10.28g; 90.03 mmole) dissolved in 40ml acetone was added and stirred for 1½ hours at 95°C. It was left to stand at room temperature for over 2 hours, The solvent was removed from the filtrate under vacuum. The solid obtained from the filtrate contained most of the oxaliplatin in a crude form.

This solid was suspended in distilled water (388ml) over a water bath at 70°C for a period of time (20 minutes) where after the remaining solid was removed by filtration. The filtrate was cooled and 0.75 molar equivalents of $Cs_2C_2O_4$ in relation to $K_2Pt(C_2O_4)_2.2H_2O$ was added. The solution was stirred for approximately 20 minutes and the precipitate which formed was filtered. The solvent was subsequently vacuum evaporated. The remaining solid was suspended in a portion of distilled water (58ml) and heated at 70°C for a period of time (20 minutes) and filtered to provide a filtrate (A).

The solid which did not dissolve contained mostly oxaliplatin with a portion of oxalate salt. This was readily purified by washing the solid with a portion (29ml) of distilled water to obtain pure oxaliplatin.

The filtrate (A) was cooled to 5°C and the precipitate filtered. The solvent was vacuum evaporated. The solid which remained contained mainly oxaliplatin and cesium and potassium oxalates. The oxalate salts were readily removed by washing 5 times with portions of distilled water (29ml) resulting in pure oxaliplatin.

All the oxaliplatin samples produced had a chemical purity >99.5% and optical purity of >99.98%. The overall yield of chemically and optically pure oxaliplatin was 15%.

<u>Example 6</u> – Method for the preparation of 2-methylthioakyl complexes of imidazole and pyridine, in this case oxalato(1-methyl-2-methylthiopropylimidazole)platinum(II).

0.68g of $K_2Pt(C_2O_4)_2 2H_2O$ was dissolved in 40 ml distilled water over a water bath at 90°C to which was added dropwise while stirring 0.638g of 1-methyl-2-methylthiopropylimidazole dissolved in 4 ml acetone. The platinum(II) solution was stirred for 1½ h at 90°C and subsequently allowed to cool overnight. The resultant precipitate was filtered and washed once with a 6 ml portion of cold distilled water and air dried in an oven at 50°C. Light yellow solid (60% yield).

<u>Example 7-</u> Method for the preparation of an aliphatic aminothioether complex of Pt(II) oxalate, in this case oxalato(1-amino-2-thiomethylethane)platinum(II).

$K_2Pt(C_2O_4)_2.2H_2O$ (0.6 mmol; 0.291g) was dissolved in 8 ml distilled water over a 90°C water bath. 32 ml dmf was added to the platinum(II) solution to form a 20:80 water:dmf solvent ratio. One molar equivalent (0.6 mmol) of the 1-amino-2-thiomethylethane ligand was dissolved in 3 ml acetone and

added dropwise while stirring whereafter the platinum(II) solution was stirred for 2 hours at 90°C. The reaction was allowed to cool overnight at room temperature and the resultant precipitate filtered, washed once with a 3 ml portion of cold distilled water and allowed to air dry in an oven at 50°C. Light yellow product 47% yield.

Example 8

The purities of the compounds of Examples 6 and 7 were determined by C, H and N analysis, infrared spectroscopy and the ligands by NMR spectroscopy. All the complexes show molecular weight peaks obtained from FAB mass spectral data which correspond with monomolecular complexes except Pt(1-methyl-2-methylthiopropylimidazole)C_2O_4 which gave only fractions.

Example 9

Anticancer testing on the complex of Example 6 was performed and compared with cisplatin under similar conditions using 5% foetal calf serum as a medium. The percentage inhibition of oxalato(1-methyl-2-methylthiopropylimidazole)platinum(II) on cervical cancer cells was 88.6% and 97.7% at 10 and 100μM solutions while the corresponding values for cisplatin are 85.4 and 95.3% respectively. A further study in which a medium containing 10mM glutathione was used the complex performed even better, viz 85% inhibition of colon cancer cells at 100μM (cisplatin 48%); 72% on breast cancer (cisplatin 10%) at 100μM solution.